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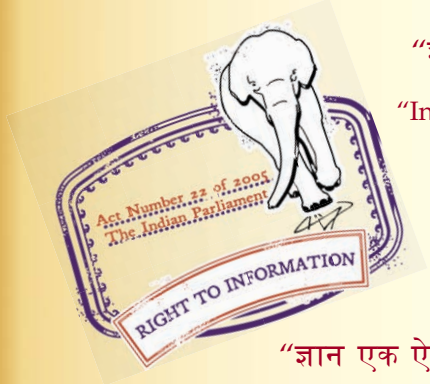
IS 3841 (2008): ß - Carotene Food Grade -Specification [FAD 8: Food Additives]



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"Knowledge is such a treasure which cannot be stolen"

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भारतीय मानक

β-कैरोटीन, खाद्य ग्रेड — विशिष्टि

(पहला पुनरीक्षण)

Indian Standard

β-CAROTENE, FOOD GRADE — SPECIFICATION

(First Revision)

ICS 67.220.20

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

June 2008

Price Group 2

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Carotene is widely distributed in nature. It derives its name from carrot (*Daucus carota*), which contains the colouring principle carotene and is an unsaturated hydrocarbon. Carotene pigment occurs in nature in three important isomeric forms, α, β and χ, out of which β-carotene is available in pure form and is also manufactured synthetically. β-carotene is a precursor of vitamin A and is fat soluble. It is also used as a food colour.

This standard is one of a series of Indian Standards for natural food colours permitted under the *Prevention of Food Adulteration Rules*, 1955.

This standard was first published in 1966 based on the then existing JECFA Specification for β-carotene. This standard is being revised taking into consideration the latest publication for the food colour issued by JECFA and also the latest specifications laid down under the US FDA and the EEC Directives. In this revision the limits for sulphated ash and subsidiary colouring matters have been included and requirements for heavy metal contaminants made more stringent to align with the international requirements.

Requirements for β-carotene, food grade have been prescribed under the *Prevention of Food Adulteration Rules*, 1955 and due consideration has been given to the rules in the formulation of this standard. Due consideration has also been given to the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to restrictions imposed under these, wherever applicable.

Description

Common Name — β-carotene

Synonyms — Cl Food Orange 5, INS No. 160a (i); Cl (1975) No. 40800

CAS Number — 7235-40-7

Chemical Names — Carotene, β, β-carotene

Class — Carotenoids

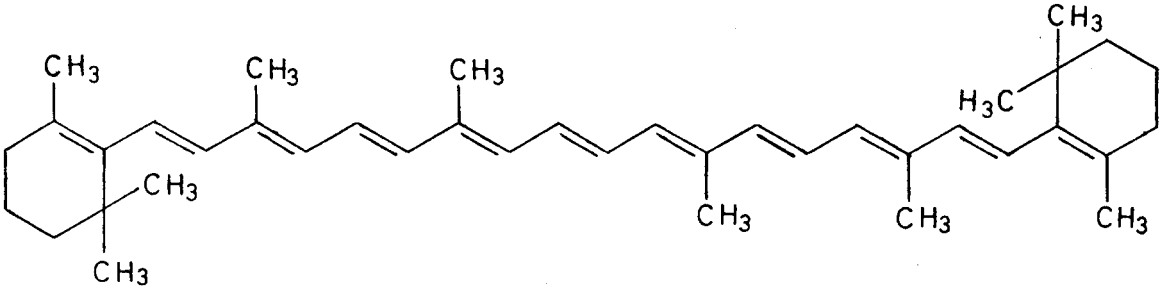
Chemical Name — All trans β-carotene

Empirical Formula — $C_{40}H_{56}$

Molecular Weight — 536.88

Solubility — Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils; soluble in chloroform

The structural formula of β-carotene is given below:



For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

β-CAROTENE, FOOD GRADE — SPECIFICATION

(First Revision)

1 SCOPE

1.1 This standard prescribes requirements and methods of sampling and test for β-carotene, food grade, both natural and synthetic.

1.1.1 This standard applies predominantly to all trans isomers of β-carotene together with minor amounts of other carotenoids.

2 REFERENCES

The following standards contain provisions, which through reference in this text constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
266 : 1993	Sulphuric acid — Specification (*third revision*)
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1994	Methods of sampling and test for food colours (*second revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)

3 DESCRIPTION

3.1 β-carotene is obtained as dark violet hexagonal prisms when crystallized from benzene-methanol solution; or as red rhombic, almost quadratic plates, from petroleum ether.

3.2 β-carotene exists as red to brownish-red crystals or crystalline powder. Diluted and stabilized forms of carotene include solutions or suspensions of carotene in edible fats or oil, emulsions and water dispersible powders. These preparations may have different cis/trans ratios.

4 REQUIREMENTS

4.1 Spectrophotometric Requirements

Ratio of absorbance of β-carotene in cyclohexane (0.2 mg per 100 ml approximately) at 455 nm and 483 nm (A_{455}/A_{483}) is between 1.14 and 1.19.

Solution of β-carotene in chloroform on addition of antimony trichloride solution shall give a dark blue colour having maximum absorption at a wave-length of 590 nm.

4.2 The material shall also conform to the requirements given in Table 1.

4.3 The product shall be processed, packed, stored and distributed under hygienic conditions in licenced premises (*see* IS 2491).

5 PACKING, STORAGE AND MARKING

5.1 Packing

The material shall be filled in amber-coloured glass container or any other suitable container sealed under an inert gas as carotene is sensitive to oxygen and light. The containers shall be such as to preclude

Table 1 Requirements for β-Carotene

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of This Standard	Clause of IS 1699
(1)	(2)	(3)	(4)	(5)
i)	Purity, percent of total colouring matters, expressed as β-carotene, *Max*	96	A	—
ii)	Subsidiary colouring matters, percent by mass, *Max*	3	B	—
iii)	Sulphated ash, percent of total colouring matters, *Max*	0.1	C	—
iv)	Arsenic (as As), mg/kg, *Max*	3	—	15
v)	Lead (as Pb), mg/kg, *Max*	10	—	15
vi)	Heavy metals, mg/kg, *Max*	40	—	16

contamination of the contents with metals or other impurities.

5.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

5.3 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or code number;
d) Net content when packed;
e) Instruction for storage;
f) Best before .... (Month and year to be given by the manufacturer); and
g) Any other requirements as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977 and *Prevention of Food Adulteration Act*, 1955 and Rules.

5.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

5.3.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in **4** of IS 1699.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

ANNEX A

[*Table* 1, *Sl No.* (i)]

METHOD FOR ASSAYING PURITY OF β-CAROTENE

A-0 METHOD

The assaying of purity of β-carotene is carried out by the spectrophotometric method.

A-1 REAGENT

A-1.1 Cyclohexane, spectrophotometric grade.

A-2 APPARATUS

A-2.1 Spectrophotometer — capable of accurate (± 1 percent or better) measurement of absorbance in the region of 350-750 nm with an effective slit width of 10 nm or less.

A-2.2 Absorption Cells, 1-cm light path.

A-3 PROCEDURE

Accurately weigh about 0.08 g (w) of sample in a 100-ml volumetric flask (V_1) and dissolve by shaking briefly with 20 ml pure, acid-free chloroform. Make sure that the solution is clear. Make up to volume by the addition of pure cyclohexane. Pipette 5.0 ml of the solution (v_1) into a 100-ml volumetric flask (V_2) and make up to volume with cyclohexane.

Similarly, dilute 5.0 ml of this solution (v_2) to 100 ml (V_3) and measure the absorbance at the absorbance maximum $(A)_{1\,cm}^{1\%}$ against cyclohexane as a blank, using 1 cm cells. Calculate the total colouring matters content according to the following formula:

$$\text{Total colouring matter, percent} = \frac{(A \times V_1 \times V_2 \times V_3)}{v_1 \times v_2 \times w \times A_{1\,cm}^{1\%}}$$

ANNEX B

[*Table* 1, *Sl No.* (ii)]

DETERMINATION OF SUBSIDIARY COLOURING MATTERS

B-1 PROCEDURE

Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 µl of this solution as a streak 2 cm from the bottom of a TLC plate (Silica gel 0.25 mm).

Immediately develop the chromatogram with a solvent mixture of 95 parts dichloromethane and 5 parts diethyl ether in a saturated chamber, suitably protected from light, until the solvent front has moved 15 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silica gel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40 ml chloroform (Solution 1).

Remove the silica gel adsorbent that contains the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered 50 ml centrifuge tube and add 20 ml chloroform (Solution 2).

Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10 ml of Solution 1 to 50 ml with chloroform (Solution 3).

Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum about 464 nm, using chloroform as blank.

B-2 CALCULATION

$$\text{Percentage of carotenoids other than carotene, percent} = \frac{A_2 \times 100}{A_3}$$

where

A_2 = absorbance of Solution 2, and

A_3 = absorbance of Solution 3.

ANNEX C

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF SULPHATED ASH

C-1 REAGENT

C-1.1 Concentrated Sulphuric acid, *see* IS 266.

C-2 PROCEDURE

Weigh accurately about 2 g of the material in a tared crucible. Ignite, gently at first, until the material is thoroughly charred, cool, moisten the residue with 1 ml of sulphuric acid and ignite gently till the carbon is completely consumed. Cool the crucible in a desiccator and weigh.

NOTE — Carry out the ignition in a place protected from air currents and use as low a temperature as possible to effect the combustion of carbon.

C-3 CALCULATION

$$\text{Sulphated ash, percent by mass} = \frac{W_1}{W_2} \times 100$$

where

W_1 = mass of the residue, in g; and

W_2 = mass of the material taken for the test, in g.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act,* 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 8 (1470).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters :

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002
Telephones : 2323 0131, 2323 3375, 2323 9402

Telegrams : Manaksanstha
(Common to all offices)

Regional Offices :		Telephone
Central	: Manak Bhavan, 9 Bahadur Shah Zafar Marg, NEW DELHI 110 002	2323 7617, 2323 3841
Eastern	: 1/14 C.I.T. Scheme VII M, V. I. P. Road, Kankurgachi, KOLKATA 700 054	2337 8499, 2337 8561, 2337 8626, 2337 9120
Northern	: SCO 335-336, Sector 34-A, CHANDIGARH 160 022	260 3843, 260 9285
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